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RESPONSE OF DIFFERENT WEED SPECIES EXTRACTS ON SEED GERMINATION, PROTEIN CONTENT AND ITS BANDING PATTERN IN DIFFERENT VARIETIES OF WHEAT.

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The effect of weed extracts was examined on seed germination, proline, total protein and its profile in ten varieties of wheat. The % reduction in germination and protein was > 50 % in all the varieties with all extracts however, positive effect was also observed. The proline was significantly stimulated in all varieties with all the extracts except PBW-154, PBW-343, PBW-443, RR-21 and UP-2382. The banding pattern of protein also influenced significantly. The SI was maximum between *A. conyzoides* x *P. hysterophorus* (52.2) in UP-2425 and minimum between *A. conyzoides* × *M. alba* (23.1) in PBW-154. On the basis of these parameters, PBW-373, PBW-443, UP-262 and UP-1109 were resistant and the inhibitory effect was maximum and minimum with *P. hysterophorus* and *C. dactylon*, respectively.

Key words: Allelopathy, proline, protein seed germination, profile, weed extracts, wheat varieties.

Plants have an ability to accumulate a wide variety of low molecular weight constituents, resulting from long metabolic pathways: the secondary metabolites called allelochemicals (Harborne 1997). These allelochemicals are released in to the environment and can have a biological effect on other organisms. This process involving secondary metabolites produced by plants, microorganisms, viruses and fungi refers to 'Allelopathy' (Rice 1984) and influences growth and development of Agricultural Crops and Biological Systems. Allelochemicals may be selective in their action or plants may be selective in their responses. All of these have inhibitory activity and most of them produce different biological lesions (Einhellig 1995). Djanaguiraman *et al.* (2005) observed physiological responses of *Eucalyptus globulus* leaf leachate on sorghum and black gram. Seed germination, shoot length, seedling dry matter and vigour index were significantly reduced and also influenced the metabolism of seedlings *viz.*, chlorophyll a, b and total, soluble protein, proline and phenol content. In general, leaf leachate increased the proline and phenol content, and decreased the chlorophyll and soluble protein contents. Verma and Rao (2006) reported that the weed extracts of *A. conyzoides, C. dactylon, P. hysterophorus* and *S. nigrum* affected the protein content and its banding pattern in different varieties of *Glycine max*, particularly the low molecular weight protein. With this background, a laboratory study was carried out to assess the effect of different weed species extracts on germination, proline content, total protein and protein profile in eight days old seedlings in different varieties of wheat commonly growing in the Tarai region of Uttarakhand and to identify resistant varieties.

MATERIAL AND METHODS

The dominant weed species *i.e.*, *Ageratum conyzoides* L., *Chenopodium album* L., *Cynodon dactylon* (L.) Pers., *Melilotus alba*

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L.*, Parthenium hysterophorus* L.*, Phalaris minor* L. and *Solanum nigrum* L. were selected and collected at the time of fruiting stage from the crop fields of G.B. Pant Univ. of Agric. & Technology, Pantnagar The extracts (10%) were prepared by soaking 10 g of crushed weed mass in 100 ml sterilized distilled water for 24 h at room temperatures, filtered through Whatman filter paper and made a final volume of 100 ml by adding distilled water and used in germination experiments. Seeds of PBW-154, PBW-343, PBW-373, PBW-443, PBW-502, RR-21, UP-262, UP-1109, UP-2382 and UP-2425 varieties of wheat (*Triticum aestivum* L.) were obtained from Seed Production Centre (SPC), Pantnagar. Healthy and uniform seeds were selected and sterilized with 0.01 % $HgCl_2$ and washed thoroughly with distilled water. The seeds were placed in Petri dishes containing two blotting papers for each variety in triplicates and moistened either with sterilized distilled water (control) or with aqueous extracts (treatment) of different weed species and kept under laboratory conditions. Radicle emergence is considered as an index of seed germination and observed at 24 h interval till eight days and expressed as per cent seed germination.

Proline (μ mol g^{-1} f. wt.) from eight days old seedlings was estimated according to the procedure of Bates *et al*. (1973). The total protein (mg g^{-1} f. wt.) was estimated by using Bradford (1976) method and the banding pattern in polyacrylamide gel electrophoresis (SDS-PAGE) by Laemlli (1970). The SDS solubilised protein samples were analyzed by one dimensional, discontinuous, vertical SDS-PAGE with 12.5 % separating and 5 % stacking gels using Tris glycine electrode buffer. Medium range molecular weight markers (Bangalore Genei, India) were used with the samples. The gel was observed on Gel Documentation System and photographed. Similarity Index (SI) was calculated on the basis of presence or absence of bands for each pair wise combination according to Sokel and Sneath (1973). ANOVA, C.V., C.D. and t test were calculated according to Snedecor and Cochran (1969).

RESULTS AND DISCUSSION

In general, the rate and % seed germination was delayed and reduced in all the wheat varieties with all weed extracts and stimulated in PBW-154 and UP-1109 with *C. dactylon*; PBW-373 with *P. minor* and PBW-443 with both *C. album* and *P. minor (Table 1, Fig. 1)*. The per cent reduction was >50 % in all the varieties and it was in all the varieties (100 %) with *M. alba, P. hysterophorus* and *S. nigrum*; nine (90 %) with *A. conyzoides;* six (60 %) with *C. dactylon* and five (50 %) varieties with both *C. album* and *P. minor*. The differences between varieties, weed species, treatments, weed species × treatments, varieties × treatments, varieties × weed species and varieties × weed species × treatment is significant (ANOVA, P<0.01). Khan (2006) studied the allelopathic potential of *Washingtonia filifera* leachates from fruits, inhibited the germination of lettuce, wheat, red cabbage and cucumber seeds. However, Pandey *et al.* (2006) reported increased seed germination in different varieties of paddy when treated with *P. hysterophorus*, *Polygonum hydropiper* and *P. minor.* The present study results indicated that the variety UP-262 was resistant and PBW-502 was most susceptible to different weed extract and followed the order: *P. hysterophorus* > *M. alba* > *A. conyzoides* > *S. nigrum* > *C. album* > *C. dactylon* and > *P. minor.*

The proline content (μ mol g^{-1} f. wt.) showed much variation among varieties with different extracts. It was significantly increased (stimulated) with all extracts, except PBW-154 with *C. dactylon;* RR-21 with both *C. album* and *C. dactylon* and PBW-343, PBW-443 and UP-2382 with *C. dactylon,* where it was reduced (Table 2 and Fig. 2). The difference

Table 1 : Effect of different weed species extracts on per cent seed germination (%) in different varieties of wheat (Mean ± SE)

Varieties	Control	Weed species						
		A. conyzoides	*C. album*	*C. dactylon*	*M. alba*	*P. hysterophorus*	*P. minor*	*S. nigrum*
PBW154	93.3±6.7	93.3±3.3	93.3±6.7	96.6±3.3	73.3±14.5	66.6±5.8	100.0±0.0	90.0±10.0
PBW343	93.3±6.7	60.0±11.6*	56.6±8.8	76.6±3.3	53.3±3.3*	30.0±5.8*	93.3±3.3	66.6±6.6*
PBW373	96.6±3.3	70.0±17.3	83.3±6.7	73.3±3.3	63.3±6.6	36.6±17.6	100.0±0.0	66.6±12.0
PBW443	96.6±3.3	80.0±5.8	100.0±0.0	93.3±3.3	66.6±12.0	86.6±8.8	100.0±0.0	70.0±15.3
PBW502	100.0±0.0	80.0±15.3	93.3±3.3	100.0±0.0	60.0±5.7*	26.6±3.3*	93.3±3.3	73.3±8.8
RR-21	100.0±0.0	93.3±3.3	96.6±3.3	96.6±3.3	83.3±8.8	73.3±14.5	100.0±0.0	83.3±8.8
UP-262	100.0±0.0	96.6±3.3	96.6±3.3	100.0±0.0	90.0±10.0	80.0±5.8	90.0±5.7	80.0±5.7
UP-1109	96.6±3.3	86.6±3.3	96.6±3.3	100.0±0.0	56.6±6.6	40.0±5.8*	93.3±6.6	90.0±10.0
UP-2382	100.0±0.0	96.6±3.3	100.0±0.0	86.6±6.7	73.3±14.5	73.3±13.3	83.3±6.6	83.3±3.3*
UP-2425	96.6±3.3	73.3±16.7	96.6±3.3	93.3±6.7	63.3±6.6*	60.0±10.0	96.6±3.3	93.3±6.6

't' test was applied. '*'significant at P>0.05.

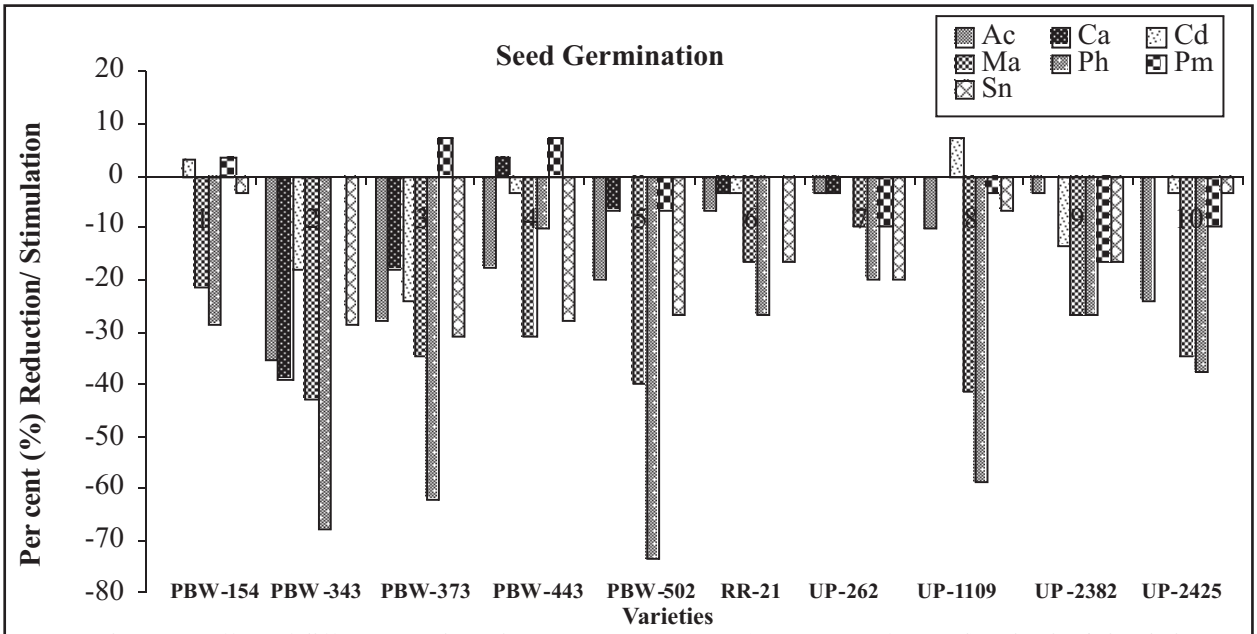

Figure 1 : Effect of different weed species extracts on per cent (percentage of control) reduction/stimulation of seed germination in different varieties of *Triticum aestivum.*

Ac = A. *conyzoides,* Ca = *C. album*, Cd = *C. dactylon*, Ma = *M. alba*, Ph. = *P. hysterophorus*, Pm = *P. minor* and Sn = *S. nigrum*

only between treatments was significant (ANOVA, P< 0.01). The proline content in relation to % seed germination was effected significantly in PBW-343 (P<0.05) and highly significantly in UP-262 (P<0.01). Djanaguiraman *et al*. (2005) reported that *E. globulus* leaf leachates increased the proline content in rice, sorghum and blackgram. In the present study, the variety UP-262 was susceptible and PBW-373 was resistant and among the extracts, *A. conyzoides* is the most deleterious and *C. dactylon* is least effective.

Table 2 : Effect of different weed species extracts on proline content (μ mol proline g^{-1} fresh weight) in different varieties of wheat.

Varieties	Control	Weed species						
		A. conyzoides	*C. album*	*C. dactylon*	*M. alba*	*P. hysterophorus*	*P. minor*	*S. nigrum*
PBW154	6.35	39.83	17.32	5.77	23.37	35.79	15.29	37.80
PBW343	4.61	72.15	59.45	3.46	21.06	59.45	10.53	34.05
PBW373	4.61	69.26	9.24	5.19	27.13	12.70	7.94	41.99
PBW443	5.77	102.74	10.39	4.62	25.39	16.16	5.92	30.16
PBW502	2.89	26.55	16.74	5.77	18.76	16.74	8.95	91.48
RR-21	6.35	20.20	5.19	4.62	19.91	18.47	9.24	40.98
UP-262	2.31	20.78	2.89	6.35	29.73	25.40	15.04	32.03
UP-1109	2.31	38.67	2.89	4.62	48.63	16.16	7.36	56.71
UP-2382	5.19	25.40	5.19	4.62	13.28	16.16	7.79	77.92
UP-2425	2.31	46.18	7.50	9.81	13.70	20.20	5.25	47.18

Cd at 5% = 0.0440; CV = 46.8098

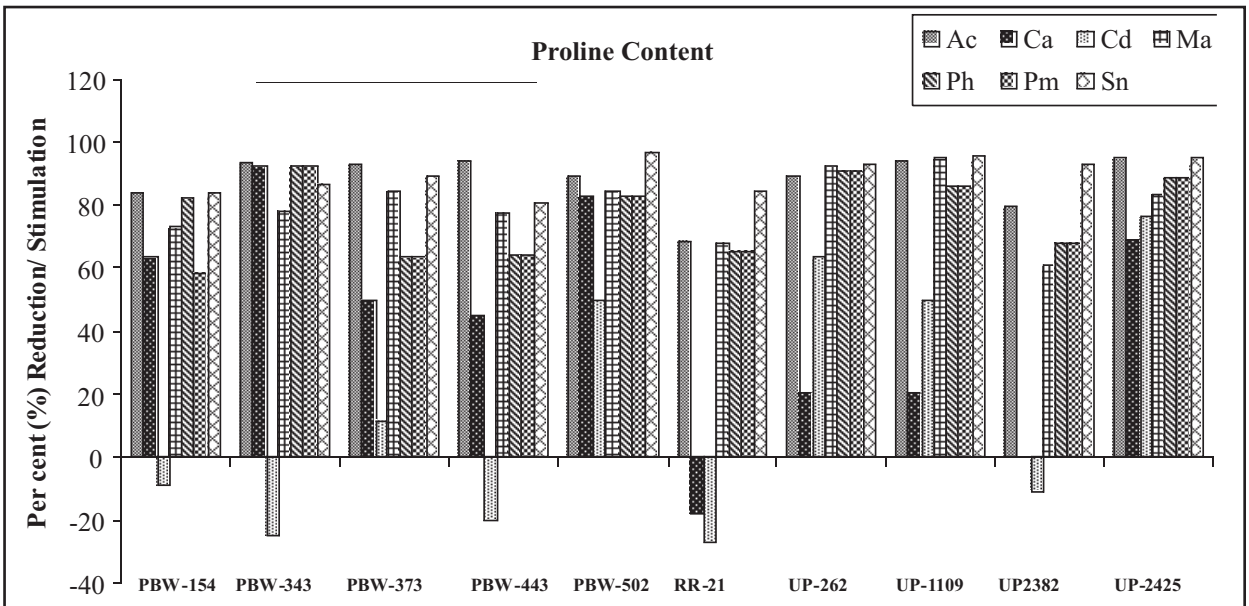

Figure 2 : Effect of different weed species extracts on per cent (percentage of control) reduction/stimulation of proline content in different varieties of *Triticum aestivum.*

Ac = *A. conyzoides*, Ca = *C. album*, Cd = *C. dactylon*, Ma = *M. alba*, Ph = *P. hysterophorus*, Pm = *P. minor* and Sn = *S. nigrum*

The total protein content (mg g^{-1} f. wt.) in different varieties also showed much variation among both varieties and extracts (Table 3). It was either decreased in UP-2382 or both increased/decreased in rest of the varieties compared to control (Table 3 and Fig. 3). Among the extracts, the % reduction was > 50 % in all the varieties (100 %) with *M. alba, P.*

Table 3 : Effect of different weed species extracts on total protein content (mg/g fresh weight) and total protein bands in different varieties of wheat.

Varieties	Control	Weed species						
		A. conyzoides	*C. album*	*C. dactylon*	*M. alba*	*P. hysterophorus*	*P. minor*	*S. nigrum*
Total Protein Content(mg/g fresh weight)								
PBW154	5.4	6.9	4.7	5.1	3.5	1.5	3.6	1.9
PBW343	5.7	3.7	4.3	6.9	2.9	1.9	3.1	3.0
PBW373	6.8	4.7	5.1	3.8	4.0	2.5	7.9	2.3
PBW443	4.9	7.1	7.0	7.1	4.8	1.1	6.7	2.8
PBW502	4.1	5.9	6.5	4.1	3.3	1.3	2.8	2.6
RR-21	4.5	5.2	5.9	4.1	2.5	2.6	5.4	2.1
UP-262	4.9	4.5	6.5	4.7	3.8	2.0	7.3	2.9
UP-1109	4.4	3.8	4.8	4.5	3.6	1.9	3.9	2.4
UP-2382	5.9	4.4	3.7	3.8	3.5	1.4	5.1	3.2
UP-2425	4.4	4.5	3.7	3.1	3.0	1.5	4.0	1.6
Cd at 5% = 0.8940; CV = 24.3766								
Total Number ofProtein Bands								
PBW154	17	14	17	15	12	15	13	11
PBW343	15	16	17	14	12	13	14	16
PBW373	12	15	14	14	16	18	16	14
PBW443	13	9	9	7	6	8	8	7
PBW502	12	11	10	11	12	11	10	10
RR-21	14	13	13	10	11	11	11	10
UP-262	10	11	11	11	10	10	11	12
UP-1109	17	16	16	16	16	16	12	15
UP-2382	14	16	13	13	13	13	13	10
UP-2425	14	11	10	11	12	12	11	13

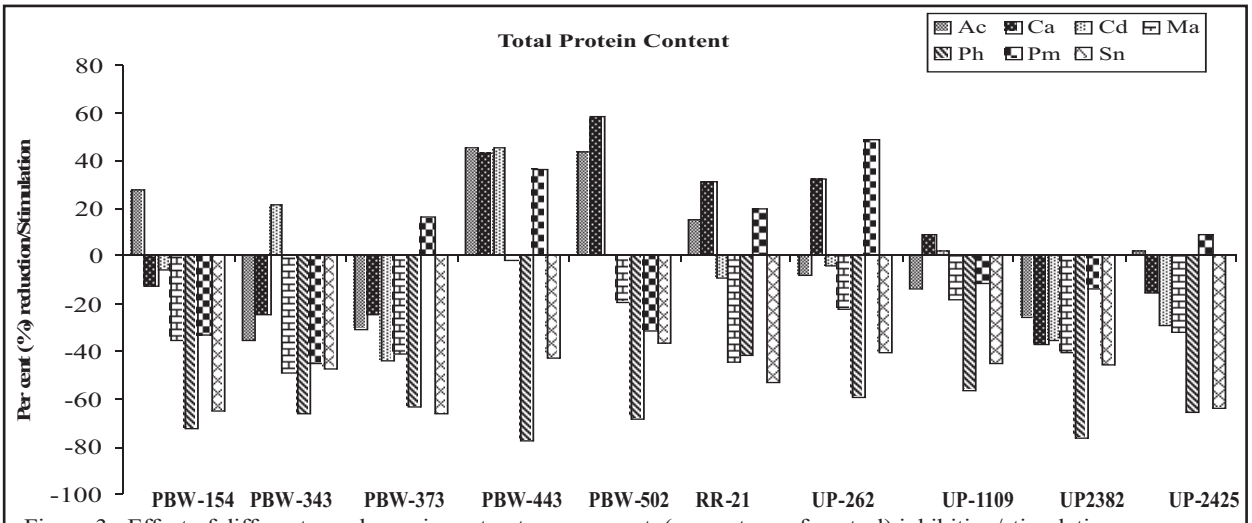

Figure 3 : Effect of different weed s pecies extracts on per cent (percentage of control) inhibition/stimulation of total protein content in different varieties of *Triticum aestivum.*

Ac = *A. conyzoides*, = Ca, *C. album*, *Cd* = *C. dactylon*, Ma = *M.alba*, Ph = *P. hysterophorus*, Pm = *P. minor* and Sn = *S. nigrum.*

hysterophorus and *S. nigrum*; six (60 %) with *P. minor* and five (50 %) with *A. conyzoides, C. album* and *C. dactylon*. Qian *et al.* (2005) observed that the soluble protein content of wheat roots was increased by the effect of volatile monoterpenoides produced by *Salvia leucophylla*. The extracts of *A. conyzoides, C. rotundus, P. minor, P. hydropiper* and *S. nigrum* reduced total protein in different varieties of wheat (Badhani *et al.* 2008). The present study indicated UP-2382 was susceptible and PBW-443 was resistant. The total protein in relation to per cent seed germination was effected significantly in UP-1109 and UP-2425 ($P<0.05$) and highly significantly in PBW-373 and RR-21 ($P<0.01$).

The protein banding pattern showed variations in treatments compared to control. The number of bands were either decreased/ increased or remain same in different varieties with different extracts (Table 3). Interestingly, they were either less or absent in low molecular weight (< 14.4 KDa) in all varieties with all extracts. In rest of the molecular ranges, the number may vary with different extracts in different varieties as compared to control. Verma and Rao (2006) reported that the extracts of four weeds affected the protein banding pattern in different varieties of *Glycine max,* particularly the low molecular weight protein. Qian *et al.* (2005) studied that *Artemisia annua* L. extract inhibited the regular karyokinesis in the meristem tissue of wheat root tips and remarkably increased soluble protein and nucleic acid. Badhani *et al.* (2008) also reported the extracts of five weeds significantly affected the banding pattern of protein in different varieties of wheat and minimum or no band(s) was present either in high (66.0 - > 97.4 KD) or low (<14.4 43.0 KD) molecular weight. The similarity index (SI) among different combinations with different varieties and extracts was maximum between *A. conyzoides* x *P. hysterophorus* (52.2) in UP-2425 and minimum between *A. conyzoides* × *M. alba* (23.1) in PBW-154. The results indicated that PBW-443 was susceptible and UP-1109 was resistant.

Thus, the present study results indicated that the influence of weed extracts on all the parameters and it is due to the presence of allelochemicals, particularly phenolics and other secondary metabolites like growth regulators, alkaloids, terpenoids and toxins (Wu *et al.* 2000). On the basis of different parameters, PBW-373, PBW-443, UP-262 and UP-1109 were resistant compared to others. Among the weed extracts, the inhibitory effect on different varieties of wheat followed the order: *P. hysterophorus* > *M. alba* > *A. conyzoides* > *S. nigrum* > *C. album* > *P. minor* and > *C. dactylon*. Natural conditions are however, more complicated than laboratory bioassays, hence, the field experiments are necessary before any final conclusions are made on allelopathic effect of these weed species.

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